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Original article

In Silico Pharmacological Evaluation and ADME Profiling of Berberine and Rosmarinic Acid as Potential Inhibitors of *Escherichia Coli Dihydrofolate Reductase*

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ABSTRACT

Antimicrobial resistance has increased the need to identify alternative antibacterial scaffolds targeting established bacterial pathways. Dihydrofolate reductase (DHFR) is an established antimicrobial target involved in bacterial folate metabolism. This study evaluated selected natural compounds as potential inhibitors of Escherichia coli DHFR (ecDHFR) using molecular docking followed by in silico drug-likeness and pharmacokinetic profiling. Ten natural compounds were screened against ecDHFR using the CB-Dock platform, and their predicted binding scores were compared with the reference inhibitor trimethoprim. The two highest-ranked compounds were subsequently evaluated using SwissADME. Rosmarinic acid and berberine demonstrated the most favorable predicted docking scores, with Vina scores of −9.0 and −8.8 kcal/mol, respectively. Both compounds showed zero violations of Lipinski's rule of five. Rosmarinic acid demonstrated low predicted gastrointestinal absorption, whereas berberine showed high predicted gastrointestinal absorption and predicted inhibition of CYP2C9 and CYP3A4. These findings identify berberine and rosmarinic acid as natural-product scaffolds worthy of further investigation against ecDHFR. However, the computational findings do not establish direct enzyme inhibition or antibacterial efficacy. Experimental enzyme inhibition, antibacterial susceptibility, pharmacokinetic, and safety studies are required to validate these predictions.

Introduction

Antimicrobial resistance represents a major challenge to the continued effectiveness of established antibacterial therapies. Escherichia coli is an important cause of community- and healthcare-associated infections, including urinary tract infections, and increasing resistance has complicated the treatment of infections caused by this organism [1,2]. Trimethoprim has historically been an important antimicrobial agent for the treatment of E. coli infections and acts primarily through inhibition of bacterial dihydrofolate reductase (DHFR), an enzyme required for the regeneration of tetrahydrofolate and consequently for essential cellular biosynthetic processes [3,4].

Resistance to trimethoprim may arise through alterations affecting the bacterial DHFR target, as well as through other mechanisms that reduce antimicrobial susceptibility. The dissemination of resistance determinants among bacterial populations further complicates the long-term utility of established DHFR-directed therapy [5]. These challenges support continued investigation of alternative chemical scaffolds capable of interacting with bacterial DHFR and potentially serving as starting points for antimicrobial drug development. Natural products represent an important source of structurally diverse molecules for drug discovery and have contributed substantially to the development of pharmacologically active compounds [6]. Their chemical diversity makes natural-product libraries particularly relevant to early-stage screening for novel interactions with established therapeutic targets. In the context of antimicrobial discovery, natural compounds have been investigated as potential inhibitors of bacterial DHFR, including polyphenolic compounds such as epigallocatechin gallate [7].

Structure-based virtual screening and molecular docking provide useful approaches for prioritizing compounds according to their predicted interactions with a molecular target before experimental testing [8-10]. However, predicted binding affinity alone is insufficient to determine whether a compound represents a suitable drug candidate. Early pharmacological evaluation should also consider physicochemical properties, drug-likeness, absorption, distribution, metabolism, and potential drug-drug interaction liabilities [11]. Integrating target-based screening with pharmacokinetic and drug-likeness prediction can therefore provide a more informative basis for prioritizing compounds for subsequent experimental investigation. The present study aimed to pharmacologically evaluate a focused library of natural compounds as potential inhibitors of E. coli DHFR using molecular docking, followed by in silico drug-likeness and ADME profiling of the highest-ranked compounds. The objective was to identify natural-product scaffolds with favorable predicted target interactions and pharmacokinetic characteristics that may justify further experimental investigation as potential antimicrobial agents.

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Methods

Protein selection

The crystal structure of Escherichia coli dihydrofolate reductase (ecDHFR) was obtained from the Protein Data Bank (PDB ID: 1RF7). The selected structure had a reported resolution of 1.80 Å and contained a well-defined ligand-binding region. The protein structure was used as the target for subsequent molecular docking analysis.

Ligand library curation

A focused library of ten naturally occurring compounds was selected for virtual screening based on their reported biological activities and availability of structural information. The compounds included baicalein, berberine, chlorogenic acid, curcumin, ellagic acid, luteolin, myricetin, quercetin, resveratrol, and rosmarinic acid. Three-dimensional structures in SDF format were retrieved from the PubChem database using the corresponding compound identifiers (Table 1).

Table 1. Natural compounds included in the virtual screening library

Natural compound name	PubChem ID
Baicalein	64982
Berberine	2353
Chlorogenic Acid	1794427
Curcumin	969526
Ellagic Acid	5281855
Luteolin	5280445
Myricetin	5281672
Quercetin	5280343
Resveratrol	445154
Rosmarinic Acid	5281792

Molecular docking

Molecular docking was performed against ecDHFR (PDB ID: 1RF7) using the CB-Dock web server, which employs the AutoDock Vina docking algorithm [9,10]. Blind docking was performed using the default parameters of the platform without predefining a binding site. This approach allowed the compounds to be screened against predicted binding cavities of the target protein. The compounds were ranked according to their predicted Vina docking scores, with more negative scores indicating more favorable predicted binding. The two compounds with the most favorable docking scores were selected for subsequent drug-likeness and ADME analysis.

Drug-likeness and ADME prediction

The drug-likeness and pharmacokinetic characteristics of the two highest-ranked compounds were evaluated using the SwissADME web server.[11] Canonical SMILES representations retrieved from PubChem were used as input. Drug-likeness was assessed according to Lipinski's rule of five, including molecular weight, lipophilicity, hydrogen-bond donors, and hydrogen-bond acceptors. Predicted pharmacokinetic characteristics included gastrointestinal absorption, blood-brain barrier permeation, P-glycoprotein substrate status, and predicted inhibition of selected cytochrome P450 isoforms.

Protein-ligand interaction analysis

The docked protein-ligand complexes generated by CB-Dock were visualized using PyMOL. The predicted binding modes were examined to identify interactions between the selected compounds and residues within the ecDHFR binding region, including hydrogen bonding, hydrophobic interactions, and π-stacking interactions where identifiable.

Data interpretation

The docking and ADME findings were interpreted as preliminary computational evidence for prioritizing compounds for further pharmacological investigation. No experimental enzyme inhibition, antibacterial susceptibility, toxicity, or clinical pharmacokinetic testing was performed in the present study.

Results and Discussion

Molecular docking and predicted ecDHFR interactions

Virtual screening of the ten selected natural compounds against ecDHFR identified rosmarinic acid and berberine as the two compounds with the most favorable predicted binding scores (Table 2). Rosmarinic acid demonstrated a Vina score of −9.0 kcal/mol, while berberine demonstrated a score of −8.8 kcal/mol. Both compounds localized within the same predicted binding cavity, which had a reported cavity volume of 2605 $Å^3$. These findings provided a preliminary structural rationale for prioritizing the two compounds for further pharmacological profiling.

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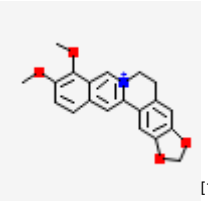
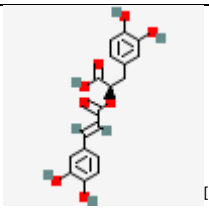
Table 2. Predicted docking scores of the two highest-ranked compounds against E. coli DHFR

Natural compound name	PubChem ID	Vina score	2D structure
Berberine	2353	-8.8	 [14]
Rosmarinic Acid	5281792	-9.0	 [15]

The predicted docking scores should, however, be interpreted as relative computational estimates rather than direct measures of inhibitory potency. Molecular docking can be useful for prioritizing compounds according to predicted ligand-target interactions, but docking scores alone cannot establish enzyme inhibition, binding kinetics, selectivity, or antibacterial efficacy [8-10]. Visualization of the docked complexes showed that both compounds occupied the ecDHFR binding region in a position broadly comparable to the reference inhibitor trimethoprim (Figure 1). Berberine demonstrated a predicted π-stacking interaction with the protein at the amino acid reside of Phe34 (Figure 2). In contrast, no clearly identifiable specific hydrogen-bond or salt-bridge interaction was observed for rosmarinic acid in the present visualization. The absence of an easily identifiable interaction in the rosmarinic acid pose does not exclude binding. Still, it indicates that the predicted pose should be interpreted cautiously and warrants experimental and/or additional computational validation. The pharmacological relevance of these findings lies in the identification of candidate natural-product scaffolds that may interact with an established antimicrobial target. However, the similarity of a predicted binding location to that of trimethoprim does not demonstrate that the compounds act through the same mechanism or possess comparable inhibitory potency. Experimental ecDHFR inhibition assays would therefore be required to determine whether the predicted interactions translate into measurable pharmacological activity.

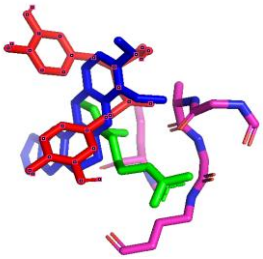


Figure 1. Predicted binding poses of trimethoprim, berberine, and rosmarinic acid within the E. coli dihydrofolate reductase (ecDHFR) binding region. The ecDHFR structure is shown in pink, trimethoprim in green, berberine in blue, and rosmarinic acid in red

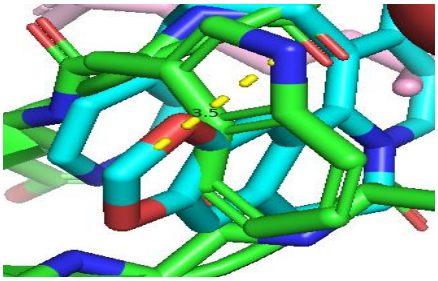


Figure 2. Predicted π-stacking interaction between berberine and E. coli dihydrofolate reductase (ecDHFR). The figure illustrates the interaction between the aromatic ring system of berberine and the corresponding aromatic residue (Phe34) within the predicted binding region

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Drug-likeness and predicted pharmacokinetic characteristics

The two highest-ranked compounds were subsequently evaluated using SwissADME to place their predicted target interactions within an early drug-development context. Both rosmarinic acid and berberine had zero violations of Lipinski's rule of five. Rosmarinic acid had a molecular weight of 360.31 Da, five hydrogen-bond donors, eight hydrogen-bond acceptors, a topological polar surface area (TPSA) of 144 $Å^2$, and a calculated logP of 1.91. Berberine had a molecular weight of 336.36 Da, no hydrogen-bond donors, four hydrogen-bond acceptors, a TPSA of 45 $Å^2$, and a calculated logP of 3.47. The predicted bioavailability scores were 0.56 and 0.55 for rosmarinic acid and berberine, respectively. The predicted pharmacokinetic profiles differed between the two compounds.

Rosmarinic acid demonstrated low predicted gastrointestinal absorption, whereas berberine demonstrated high predicted gastrointestinal absorption. Neither compound was predicted to permeate the blood-brain barrier or act as a P-glycoprotein substrate in the present analysis. Rosmarinic acid was not predicted to inhibit the evaluated CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4 isoforms. In contrast, berberine was predicted to inhibit CYP2C9 and CYP3A4, indicating a potential for pharmacokinetic drug-drug interactions that would require experimental confirmation. These findings illustrate why predicted target affinity should not be considered in isolation when prioritizing compounds for pharmacological development. Rosmarinic acid demonstrated the more favorable docking score but also showed low predicted gastrointestinal absorption and a relatively high TPSA. High polarity can reduce passive membrane permeability and may contribute to limited absorption [12].

Conversely, berberine demonstrated high predicted gastrointestinal absorption and a lower TPSA but was associated with predicted CYP2C9 and CYP3A4 inhibition. These differences represent a pharmacological trade-off between predicted exposure characteristics and potential metabolic interaction liabilities. Rosmarinic acid has been investigated for a range of biological and pharmacological activities, supporting its consideration as a natural-product scaffold for further pharmacological investigation [14]. Berberine has likewise demonstrated diverse pharmacological activities in experimental studies, although these properties should not be interpreted as evidence of ecDHFR inhibition in the present study [15]. Importantly, the ADME results generated in the present study are computational predictions rather than experimentally determined pharmacokinetic measurements. Therefore, the predicted gastrointestinal absorption and CYP inhibition profiles should be regarded as parameters for lead prioritization rather than evidence of clinical oral bioavailability or established drug-drug interaction risk.

From a pharmacological perspective, predicted target affinity alone is insufficient for prioritizing a compound as a potential drug candidate. A compound must also possess physicochemical and pharmacokinetic characteristics that permit adequate exposure to the intended target while minimizing unfavorable drug-drug interactions and safety liabilities. Therefore, the compounds demonstrating favorable predicted interactions with ecDHFR were subsequently evaluated for drug-likeness and ADME-related properties. This integrated approach allowed target-level binding predictions to be considered alongside characteristics relevant to early-stage drug development [6,8,11].

Pharmacological significance and implications for drug discovery

The integrated findings provide a preliminary pharmacological basis for prioritizing berberine and rosmarinic acid for further investigation against ecDHFR. The rationale for this prioritization is not based solely on docking scores but on the combination of predicted target interaction, drug-likeness, and pharmacokinetic characteristics. This integrated approach is consistent with the early stages of structure-based drug discovery, in which target engagement and physicochemical and pharmacokinetic properties are considered together when selecting compounds for experimental evaluation [6,8,11]. The comparison with trimethoprim provides a pharmacological reference because bacterial DHFR is an established antimicrobial target. However, the present computational findings cannot determine whether either natural compound would retain activity against trimethoprim-resistant E. coli or whether the predicted interactions would result in selective inhibition of bacterial DHFR.

Further studies should therefore evaluate direct ecDHFR inhibition, antibacterial susceptibility, and selectivity against relevant host DHFR systems before therapeutic potential can be considered. The two compounds also illustrate different development considerations (Table 3). Rosmarinic acid showed the most favorable predicted docking score but low predicted gastrointestinal absorption, suggesting that formulation or chemical optimization may be relevant if systemic exposure is required. Berberine showed high predicted gastrointestinal absorption but also predicted inhibition of CYP2C9 and CYP3A4, which may represent a potential metabolic interaction liability. These findings emphasize the importance of considering pharmacokinetic properties alongside molecular target affinity during early lead selection. The drug-likeness and ADME properties of Rosmarinic acid and berberine were evaluated via the SwissADME web server to assess their therapeutic potential (Tables 3 and 4).

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Table 3. Predicted pharmacokinetic characteristics of berberine and rosmarinic acid

Parameter	Rosmarinic Acid	Berberine
GI absorption	Low	High
BBB permeation	no	No
P-gp substrate	no	No
CYP1A2 inhibitor	no	No
CYP2C19 inhibitor	no	No
CYP2C9 inhibitor	no	Yes
CYP2D6 inhibitor	no	No
CYP3A4 inhibitor	no	Yes
Log Kp (skin permeation, cm/s)	-6.28	-5.78

CYP: Cytochrome P450.

Drug-likeness Profile: both compounds demonstrated favorable molecular characteristics for oral administration, exhibiting zero violations of Lipinski's rule of five (Table 4).

Table 4. Predicted drug-likeness characteristics of berberine and rosmarinic acid

Parameter	*Rosmarinic acid*	*Berberine*
molecular weight	360.31	336.36
H- bond donors	5	0
H-bond acceptors	8	4
TPSA (Å^2)	144	45
Lipinski's violations	0	0
cLogP	1.91	3.47
Bioavailability score	0.56	0.55

cLogP: Calculated octanol-water partition coefficient; TPSA: Topological Polar Surface Area.

Limitations

This study has several limitations. First, all findings were generated using computational methods and therefore do not establish direct inhibition of ecDHFR or antibacterial activity. Second, molecular docking scores represent predicted ligand-protein interactions.

Conclusion

This study used an integrated in silico pharmacological approach to prioritize natural compounds as potential inhibitors of Escherichia coli dihydrofolate reductase. Rosmarinic acid and berberine demonstrated the most favorable predicted docking scores among the ten screened compounds, while both compounds showed zero violations of Lipinski's rule of five. Their predicted pharmacokinetic profiles nevertheless differed substantially: rosmarinic acid showed low predicted gastrointestinal absorption, whereas berberine showed high predicted gastrointestinal absorption together with predicted inhibition of CYP2C9 and CYP3A4. These findings demonstrate the importance of considering target interaction together with drug-likeness and ADME characteristics during early-stage pharmacological lead selection. Berberine and rosmarinic acid should therefore be regarded as candidates for further investigation rather than confirmed antimicrobial agents. Experimental evaluation of ecDHFR inhibition, antibacterial activity, selectivity, pharmacokinetics, and safety is required to determine whether the predicted properties translate into meaningful pharmacological activity.

Conflict of interest. Nil

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